Marine Environmental Research 163 (2021) 105234

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: http://www.elsevier.com/locate/marenvrev

Integrative assessment of ecological responses and chemical contamination of urban wastewater outfalls on soft bottom sediments of an estuarine system

Inés G. Viana [a,b,*], Raimundo Blanco [a,b], Óscar Nieto [c], Alberto Molares [c], Ricardo Beiras [a,b]

[a] Department of Ecology and Animal Biology, University of Vigo, 36310, Vigo, Galicia, Spain
[b] ECIMAT-CIM, University of Vigo, Illa de Toralla S/n, 36331, Vigo, Galicia, Spain
[c] Department of Analytical and Food Chemistry, University of Vigo, 36310, Vigo, Galicia, Spain

ARTICLE INFO

Keywords:
Galician rias
Sea urchin bioassay
Benthic indicators
Metals
Organic compounds
Marine pollution

ABSTRACT

Sewage outfalls are one of the main anthropogenic impacts to coastal systems. Linking the chemical analysis in sediment, and changes in the ecological responses is one of the main tasks in ecosystem assessment for the protection of the marine environment. A three-tiered approach to evaluate the impact of the sewage of a recently built and obsolete Urban Waste Water Treatment Plants (UWWTPs) was performed. The sewage from each UWWTP differentially affected surrounding sediments. Macrobenthic community changes were influenced by the chemical composition in the sediments but also by grain size and organic matter. Abundance of the different families of the macrobenthic communities were correlated with most of the chemicals analyzed. On the contrary, toxicity bioassays were correlated with Zn and PCB concentrations but not with environmental variables. The toxicity tests linked the effect of chemical contamination on macrobenthic communities. A joint assessment of both chemical concentrations and their ecological effects is recommended.

1. Introduction

Wastewater discharges are one of the main sources of pollution in coastal areas significantly affecting these systems worldwide. Based on this, the European Union (EU) established the Urban Wastewater Treatment Directive (91/271/EEC) (UWWTD) to protect the environment from the adverse effects of insufficiently treated wastewater discharges. Therefore, member states of the EU are required to implement improvements on the urban wastewater treatment plants (UWWTPs) to ensure quality of sewage effluents. The UWWTD is constantly under evaluation as populations in several urban nuclei continue increasing and many UWWTPs become obsolete. Furthermore, the improvements in the depuration processes continue happening, such as the development of secondary and tertiary treatments, which are focused on the removal of residual organics, inorganic nutrients, such as nitrogen (N) or phosphorus, and coliforms. Therefore, this Directive recognized the upgrade and the general need for the establishment of improved treatments of urban wastewater to prevent environmental impact.

Monitoring is necessary to assess the efficiency of the UWWTPs upgrades in furthering the protection in coastal waters. In this way, sewage monitoring has been traditionally assessed by means of chemical analysis of a list of compounds on different environmental matrices (Beiras et al., 2003a). But the assessment of anthropogenic impacts in coastal systems is complex as there are many confounding factors, such as sediment characteristics, the natural occurrence of many chemicals or the presence of not-known contaminants in the sewage, the so-called emergent contaminants (e.g. Kupper et al., 2006). Moreover, the presence of certain contaminants does not necessarily mean that they are bioavailable to organisms (Bellas et al., 2011; Beiras et al., 2012a). Therefore, there is no simple relationship between the presence and levels of pollutants, and their ecological effects on biological communities.

In this way, different biological responses have been proposed to detect ecological effects established on soft bottoms. Benthic macroinvertebrate communities live in direct contact with the sediment, and wastewater acts as a stress factor, which is ultimately manifested by changes in their biodiversity (Pearson and Rosenberg, 1978; Warwick et al., 1990; Del-Pilar-Ruso et al., 2007, 2008). Therefore, macroinvertebrate community changes have been widely used as indicators of diverse anthropogenic impacts as different taxa show different

* Corresponding author. Department of Ecology and Animal Biology, University of Vigo, 36310, Vigo, Galicia, Spain.
E-mail address: ines.viana.gonzalez@uvigo.es (I.G. Viana).

https://doi.org/10.1016/j.marenvres.2020.105234
Received 2 September 2020; Received in revised form 19 November 2020; Accepted 30 November 2020
Available online 3 December 2020

sensitivities to pollution (Dauvin et al., 2010; Del-Pilar-Ruso et al., 2010; de-la-Ossa-Carretero et al., 2012a). Marine invertebrate embryo-larval bioassays are also well-known techniques among those frequently used in coastal monitoring because of their sensitivity, ecological relevance and cost-effectiveness (Durán and Beiras, 2010). But studies focused on linking chemical contamination and biological responses, such as toxicity and macrobenthic community changes, are scarce (Mucha et al., 2003; Marin et al., 2007; Beiras et al., 2012b). While there is also a lack of reports on the comparative effect of differently treated sewage, as obsolete and new UWWTPs, or the impact of upgrades (Del-Pilar-Ruso et al., 2010).

The aim of this study was to evaluate the differential impact of sewage from two different outfalls, an obsolete and a new-built UWWTP, to the soft bottoms of Ria de Vigo (NW Iberian Peninsula) by the analysis of the chemical composition of the sediment, sediment toxicity bioassays, and changes in benthic fauna communities.

2. Material and methods

2.1. Study area

The Ria de Vigo is a highly populated tidal inlet in Galicia, NW Iberian Peninsula, located between 42°09' and 42°21′N, and 8°36′ and 8°54′W, being the most southern of the Galician Rias Baixas. It has 35 km long and a mean width over 4 km, while its mouth, with 35 km width, is oriented towards the West. The depth varies from 52 m in the mouth to intertidal sandbanks in the inner estuary. The ria has little continental supplies of seven water-streams and a river, compared to the oceanic influence. In this way, the ria can be divided in three zones according to the degree of continental or oceanic influence. The innermost zone shows the characteristics of a typical estuary and is influenced by the river Oitavén-Verdugo, with a mean annual flow of 13 m^3 s^{-1} (Ríos et al., 1992). The middle zone is under the influence of both continental and oceanic contributions. And, finally, the outer zone, where our sites were located, is under dominant oceanic influence.

The southern margin of the ria is the most populated area, and the city of Vigo, with 300,000 inhabitants, is the most populated city. This margin also sustains most of the industrial activities of the area, with a car factory, shipyards, and a harbor based on seafood and goods. With the aim of granting the optimal depuration of the wastewater to the current and predicted population of the city of Vigo and in accordance with the objectives of the UWWTD, in the year 2008, the construction of a new UWWTP with a tertiary system and adapted to treat sewage of up 800,000 inhabitants was approved. In January 2017, the new UWWTP started working, at the same time that the old UWWTP stopped its activity. The main improvements of the new UWWTP lay on the novel biofiltration, hydrolysis and sludge treatments, that removes the N and organic matter (OM) from the sewage (8 m^3 s^{-1}), and on the construction of a new submarine sewage outfall of ~3800 m long, with 776 m built on the land, and 3016 m under water, therefore sitting the outfall

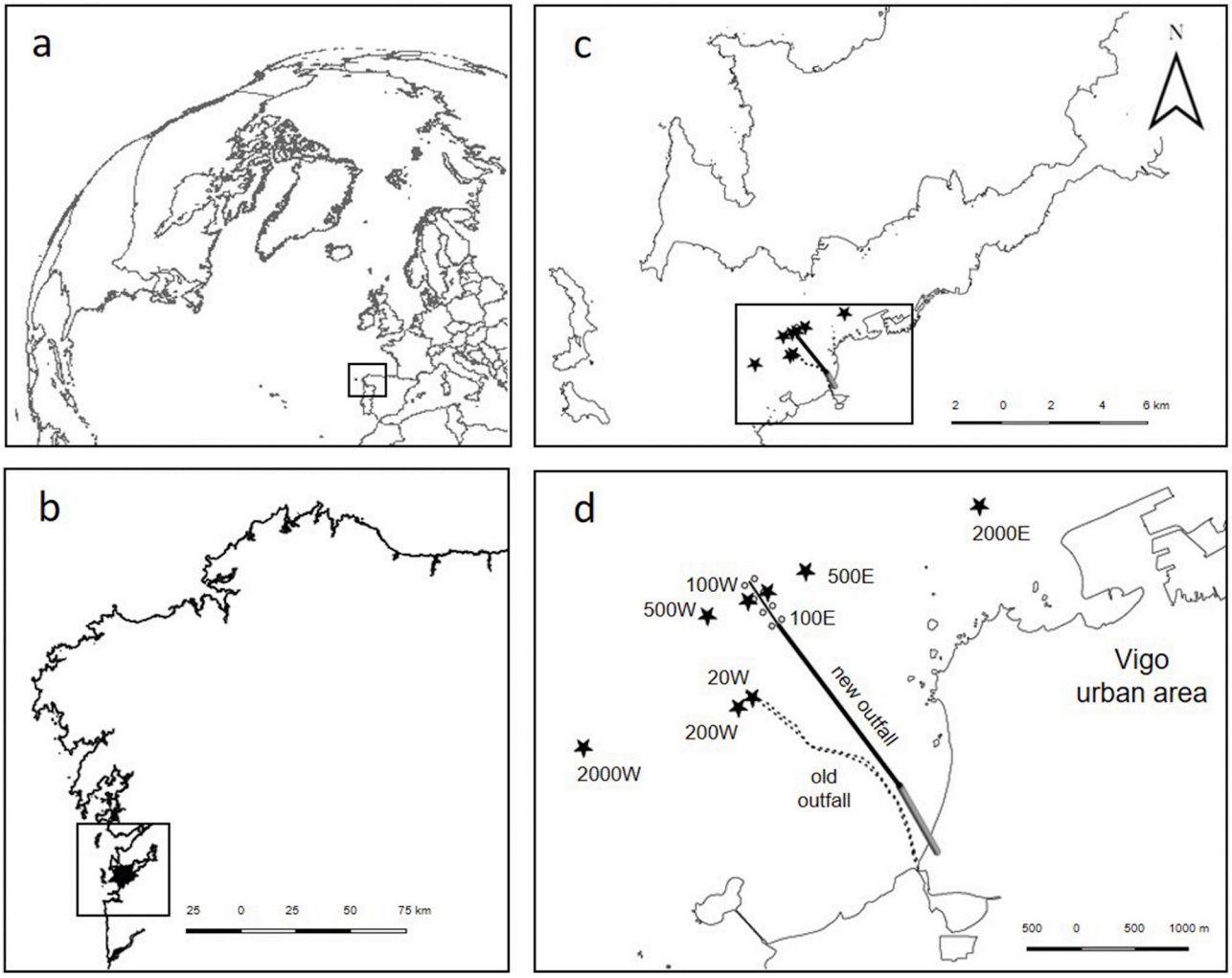

Fig. 1. Map and location of the study area at Ria de Vigo (NW Iberian Peninsula) (panels a and b). Sampling sites (black stars) at Ria de Vigo (panel c) in the proximities of the old outfall (dashed grey line) and the new outfall (black line) (panel d) of the wastewater treatment plants from Vigo urban area.

further apart from the coast (42°13′45.1″N, 8°47′49″W). The new outfall has 2 m diameter and is equipped with a diffuser section located along the final section of 335 m long at ~37 m depth. On the contrary the old outfall ($1.9 \pm 0.2\ m^3\ s^{-1}$) was sited at ~28 m depth and closer to the coastline (42°13′2.7″N, 8°47′47″W). Therefore, the new outfall drains the residues in the central channel of the ria (Fig. 1) at a deeper location than the old outfall. The new outfall is designed to maximize mixing and dilution of the effluent towards the open ocean.

2.2. Sampling

Samplings were carried out by boat (OV Kraken) in the sites shown in Fig. 1. A total of 8 sites located in the surroundings of both the new and old sewage outfalls (or sewage outlet) were sampled. Two sites were located at the western side of the old outfall, at 20 and 200 m distance from the diffuser. While the other 6 sites were located at both sides (East and West) of the diffuser section of the new outfall at increasing and equidistant distances, 100, 500, and 2000 m. The sites located at 2000 m distance from the discharge point were considered as beyond the influence of the discharges. Sampling sites were chosen based on the distances to the outfall, similar sediment composition and depth characteristics, and the direction of flume of the old outfall as observed on satellite photographs. Samplings were carried out at two consecutive years, in November 2016, to evaluate the impact of the sewage of the old UWWTP and the impact of the construction of the new outfall; and December 2017, to evaluate the impact of the outfall of the new UWWTP and the potential recovery of the soft-bottoms close to the old UWWTP.

Four sediment samples were collected at each site using a Van Veen grab with a sampling area of 0.054 m^2. Three samples were used for soft benthic infaunal community characterization and one sample was used to analyze the grain size composition, total organic carbon (TOC), carbonates, OM, N isotopic composition, chemical composition, and to obtain the elutriate for toxicity bioassays. The replicate samples were taken randomly as the boat was not anchored (Dauvin, 2000). Samples were transported in darkness in polyethylene trays to the Toralla Marine Science Station (ECIMAT, CIM, University of Vigo) for processing.

2.3. Sample processing

Once at the laboratory, the volume of each sample was mixed and divided in three aliquots: Sediment intended for OM, TOC, carbonate content, grain size, elemental and N isotopic composition were dried at 60 °C in an air forced oven until constant weight. Chemical analyses of polycyclic aromatic hydrocarbon (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were stored at −80 °C and subsequently freeze-dried. Sediment used for the toxicity bioassays was stored at 4 °C in the dark for maximum 7 days. All freeze-dried and dried samples were ground to a fine powder with a porcelain mortar and pestle and stored until analysis.

2.4. Chemical analysis in sediments

Grain size analysis–Approximately 1 g DW of sediment was digested with H_2O_2 during two days and gently shacked every 6 h until no effervescence was observed. The sample was then cleaned by vortexing and centrifuging with 30 ml of distilled water in three consecutive steps. The samples were vortexed in 10 ml of $(NaPO_3)_6$ and preserved in the darkness until analyzed the following week. Characterization of the particle size was obtained using a Beckman Coulter LS 13,320 analyzer. The following granulometric fractions were considered: coarse sand (>0.5 mm), fine sand (63 μm-0.5 mm) and silt/clay (<63 μm).

Analysis of TOC, N, carbonate content and OM– Aliquots of 3 g DW were used to estimate the TOC, N and carbonate content. Inorganic carbon in the sediments was removed by exposing the samples to HCl (37%) vapors for 72 h. Samples for TOC were analyzed with an elemental microanalyzer Fisons Carlo Erba EA1108 coupled to LECO CNS 2000. The OM content was measured from the previously dried samples, and incineration in furnace at 450 °C for 24 h.

Analysis of N isotopic composition–Dried aliquots were placed in tin capsules and introduced into an isotope-ratio mass spectrometer (DeltaV Advantage: Thermo Scientific) via an element analyzer (FlashEA1112: ThermoFinnigan). Isotopic results are expressed in δ notation in units per mil (‰):

$$\delta^{15}N\ (‰) = [(R_{sample}\text{-}R_{standard})/R_{standard}]\ x\ 10^3$$

where R was $^{15}N/^{14}N$. The standard used was the atmospheric air. In each analytical sequence USGS 40 (−4.52‰), USGS41a (+47.55‰), IAEA-N-1 (+0.4‰), IAEA-N-2 (+20.3‰) and USGS-25 (−30.4‰) were used as secondary standards. Acetanilide was used as the reference standard for quantifying the precision of the analysis (±0.15‰, n = 10).

Analysis of elements and trace elements–The samples for Cd and Hg were digested following Beiras et al. (2003b). The total Hg was determined by cold vapour atomization using a PerkinElmer FIMS 400 equipment ($SnCl_2$ as reducing agent). Cd was determined by electrothermal atomic absorption spectrometry (PerkinElmer SpectrAA-800 equipment). The Al, Ca, Cl, Fe, K, Mg, Na, P, S, Si, Ti Ba, Br, Cr, Cu, I, Mn, Ni, Pb, Rb, Sr, Y, Zr and Zn were determined by Sequential X-ray spectrometer (Siemens SRS 3000, Siemens AG, Germany). The quality of the measurements was assessed by analyzing PACS-2 reference material (National Research Council, Canada).

Analysis of PAHs, PCBs and OCPs–The samples for PAHs analysis followed the methodology detailed in Nieto et al. (2006). Seventeen PAHs, including low molecular weight PAHs (LMWPAHs): acenaphthene (Ace), acenaphthylene (Aci), anthracene (Ant), fluorene (Flu), naphthalene (Naph), and phenanthrene (Phe); and high molecular weight PAHs (HMWPAHs): benz[a]anthracene (BaA), benzo[b]fluoranthene (Bbf), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (BghiP), benzo[a]pyrene (BaP), benzo[e]pyrene (BeP), chrysene (Chr), dibenz[a,h]anthracene (DahA), fluoranthene (Fla), indeno[1,2,3-c,d] pyrene (Ipy) and pyrene (Pyr) were determined.

The analysis of PCBs and OCPs followed the method described in detail by Smedes and de Boer (1997). The total PCB concentrations of the seven individual congeners (7CBs, IUPAC No. 28, 52, 101, 118, 138, 153 and 180) considered as priority marine pollutants by international agencies (Wells and Cofino, 1997) were studied together with congeners 18, 31 and 44. The analyzed OCPs included γ-lindane, α-lindane, β-lindane, heptachlor, heptachlor-epoxide, isobenzan, α-endosulfan, hexachlorobenzene (HCB), aldrin, dieldrin, endrin, and isodrin. The total DDTs were represented by the sum of pp'DDE, pp'DDD and pp'DDT.

The determination of all these chemicals was performed by Agilent gas chromatograph (GC Agilent 7820) equipped with a column DB-XLB, and coupled to a MS Agilent 5975. 1 μL of sample was injected in the splitless mode and chemicals were detected in the SIM/Scan mode. Deuterated pyrene-D10 and congener 209 were used as surrogates for PAH and PCB, and OCP analysis respectively. Anthracene-D10, benz[a] anthracene D12 and tetrachloro m-xylene were used as internal standard forms for PAH, PCB and OCP determination. Calibration curves were assessed with PAH-Mix 9 and BeP; PCB-Mix 19, PCB 105, PCB 156, PCB 187; and Pesticide-Mix 20 (Dr. Ehrenstorfer GmbH, Augsburg, Germany) for PAH, PCB and OCP determination respectively. Limits of detection (LOD) for PAHs and PCBs varied between 1 and 10 $\mu g\ kg^{-1}$ for LMW (e.g. LMWPAHs or congener CB28) and HMW (e.g. HMWPAHs) compounds respectively. While LOD for OCPs varied between 20 and 60 $\mu g\ kg^{-1}$ for LMW and HMW compounds respectively. The quality of the analytical measurements was assessed with NIST SRM 1491b reference material. Repeatability of all organic chemical analyses was assessed by the triplicate measure of all samples and reported as the standard deviation of the mean values.

2.5. Embryo-larval bioassays

The toxicity of sediments was assessed by means of sediment elutriate analysis by sea urchin (*Paracentrotus lividus*) embryo bioassay following standard methods (Fernández and Beiras, 2001; Beiras et al., 2003b, 2012a).

For each sample, two toxicity parameters were obtained: the percentage net response (PNR), which is the control-corrected size increase in the undiluted elutriate, and the toxic units (TU), which takes into account all the dilutions tested. Toxic units were calculated as TU = $100/EC_{50}$, where EC_{50} is the theoretical dilution of the elutriate causing an inhibition of 50% in the larval development. Following the ecotoxicological assessment criteria proposed by Durán and Beiras (2010), the sediments were classified into five categories of ecological status in line with the European Water Framework Directive (WFD) namely high, good, moderate, poor and bad (see also Beiras and Durán, 2014).

2.6. Soft macrobenthic infaunal communities

The samples were sieved through a 2 and 1-mm mesh screen on board, fixed in 4% formaldehyde and stored. In the lab, the macrofaunal samples were sorted and the specimens were identified at the lowest possible taxon level. However, as analysis of benthic community at high taxonomic levels have been shown reliable results in studies of anthropogenic effects of benthic infauna (de-la-Ossa-Carretero et al., 2012b) data analysis was performed on data to family classification level. Descriptive univariate measures of macrobenthic communities were calculated for each site at each sampling year: number of families (S), total abundance (N, individuals m^{-2}), Margalef's species richness (d), Pielou's evenness index (J′), and Shannon-Wiener diversity index (H′, log_2) were estimated in each site. BOPA index (Dauvin and Ruellet, 2007) was calculated for each site:

$$\text{BOPA index} = \log[f_{Polychaeta}/(f_{Amphipoda}+1)+1]$$

where $f_{Polychaeta}$ is the opportunistic Polychaete proportion of total benthic fauna (0–1) and $f_{Amphipoda}$ is the amphipod proportion of total benthic fauna excluding the *Jassa* genus (0–1). The BOPA index ranges from 0, where there are no opportunistic polychaetes, to 0.30103, where there are only opportunistic polychaetes, reflecting the most disturbed situation. Thresholds presented by Dauvin and Ruellet (2007) for BOPA index, in line with the ecological classification proposed by the WFD, were used to establish the ecological status of each site.

2.7. Data analysis

To first compare concentrations of chemicals across sites with different sediment characteristics, the concentrations of organic chemicals (i.e., PAHs, PCBs and OCPs) were normalized to 2.5% TOC, while the concentrations of metals (i.e. Cd, Cr, Cu, Hg, Pb and Zn) were normalized to 5% Al (OSPAR Commission, 2010). To interpret the significance of chemical concentrations, normalized data was compared to background assessment concentrations (BAC) and environmental assessment criteria (EAC) when possible (OSPAR Commission, 2010) and to other sediment quality criteria (SQC) (Long et al., 1995; MacDonald et al., 1996; Bellas et al., 2011).

Following to this representation, ordination and relationships between sediment data and biological responses, based on sea-larval urchin bioassays and macrobenthic community structure, were done. Analysis of macrobenthic infaunal communities, environmental and chemical data was carried out through non-parametric multivariate techniques (Field et al., 1982). A similarities matrix between sites was constructed by means of the Bray-Curtis similarity coefficient on previously square-rooted transformed data on taxa abundance to down weight the contribution of the most abundant taxa. For environmental and chemical variables, normalized Euclidean distances were used and data were log transformed. From these matrices, classifications of the sites were performed by hierarchical clustering analysis based on the group-average sorting algorithm, as well as ordinations by means of non-metrical multidimensional scaling (nMDS) (Kruskal and Wish, 1978). The PRIMER v6 (Plymouth Routines in Multivariate Ecological Research) software package was used in these analyses (Clark and Warwick, 1994).

Pearson coefficient analysis (Zar, 2007) was used as an exploratory tool to establish correlations among environmental and chemical variables, and biological responses. This analysis was done by SPSS software (IBM SPSS Statistics for Windows v.24, Armonk, NW, USA). Data was also previously transformed to meet the assumptions of this analysis.

Following this procedure, canonical correspondence analysis (CCA) enabled to show the patterns of variation in macrobenthic infaunal community composition that can be best explained by the environmental and chemical variables (Braak, 1986). The *Vegan* package (Oksanen et al., 2019) under R (R Studio Team, 2018) was used to perform this analysis. To run the CCA, taxons present at just one site or not considered to be representative were excluded from the analysis. Environmental and chemical variables that were redundant or not correlated with biological responses were also excluded. The forward selection was employed to detect which variables explained the most variance in the macrobenthic infaunal community composition data. The following variables were considered in these analyses: OM (%), silt/clay (%), Cu, Hg, Pb, Zn, Σ LMWPAHs, Σ HMWPAHs, Σ 7CBs, and Σ OCPs.

3. Results

3.1. Environmental variables

The characteristics of the sediments sampled are shown in Table 1. Sites were soft bottoms of muddy nature (silt/clay) with low percentage of fine sand (Table 1). Percentages of silt/clay were higher in sites closer to the old outfall, showing in the year 2016 a small percentage of coarse sand that was not observed in 2017. Coarse sand was also observed in other stations, as 100E in 2016 or 500E and 2000E in 2017 (Suppl. Fig. 1).

OM varied from values of 5.9% in the surroundings of the old outfall to 9.7% in the closest sites to the new outfall (100E and 100W) and 9.6% at the furthest sites (2000E and 2000W, Table 1). The greatest change in the OM content between years was observed at 100W site, with an increase of 1.2% between both years. Contrary, at 20W, the closest site to the old outfall, the OM content decreased in 2017, when the old UWWTP was not working. TOC also differed between sites closest to the old outfall and further sites. While in the surroundings of the old outfall, TOC content was 2.6%; in the closest sites to the new outfall, values increased up to 3.7%, and this content was maintained across the other sites. Highest carbonate contents were recorded in the proximities to the old outfall (2.3–3%) while contents in the proximities to the new outfall and in the other sampling sites within the ria varied between 0.3 and 0.7% (Table 1).

N content in sediment samples were low (Table 1), varying from 0.2%, in the closest sites to the old outfall, to maximum values of 0.4%, observed in sites 500E and 2000E, which are towards the inner part of the ria. The $\delta^{15}N$, that is a tracer of the intensity and spatial extent of anthropogenic N inputs, showed the highest values at 20W and 200W, the closest sites to the old outfall, in 2016 (6.4 and 5.8‰ respectively). While the values at the closest sites to the new outfall, 100E and 100W, in 2017 were 5.5 and 5.4‰ respectively. Nevertheless, these latter values were not as enriched as the values observed in the proximities to the old outfall, or at the inner sites of the ria (ca. 5.6‰).

In summary, two different groups of sites can be distinguished based on the silt/clay, OM, carbonate, TOC and N content (nMDS, minimum stress = 0.01, n = 16). The first group is formed by the sites closest to the old outfall (20W and 200W), and the second, by the rest of the sites

Table 1
Sampling sites at Ria de Vigo (Fig. 1), its location (N–W coordinates) and the distance (m) relative position to the closest outfall, depth (m), and sediment characteristics at each site as percentage of silt/clay (silt/clay), percentage of organic matter (OM), percentage of carbonate (Carbonate), percentage of total organic carbon (TOC), percentage of nitrogen (N) and the ratio of nitrogen stable isotopes ($\delta^{15}N$, ‰) in 2016 (first value) and 2017 (second value).

Site	Location (N–W)		Closest outfall	Distance and relative position to the outfall	Depth	silt/clay	OM	Carbonate	TOC	N	$\delta^{15}N$
EL09	42° 13.06′	−8° 47.88′	old	20W	27.85	42.37	6.11	2.51	2.47	0.18	6.42
						31.14	5.82	2.93	2.22	0.19	5.56
EL07	42° 13.01′	−8° 47.97′	old	200W	28.77	39.19	5.82	3.02	2.81	0.17	5.80
						30.57	5.93	2.31	2.78	0.20	5.56
EL01	42° 13.58′	−8° 47.77′	new	100E	36.98	27.46	9.98	0.68	3.74	0.33	5.54
						18.92	10.06	0.35	3.92	0.32	5.50
EL04	42° 13.54′	−8° 47.91′	new	100W	36.85	16.06	8.84	0.47	3.49	0.30	5.38
						19.91	10.00	0.33	3.77	0.32	5.46
EL02	42° 13.68′	−8° 47.52′	new	500E	37.77	17.32	11.21	0.3	4.09	0.36	5.68
						23.09	10.42	0.64	3.87	0.34	5.40
EL05	42° 13.46′	−8° 48.18′	new	500W	37.03	13.09	9.90	0.31	3.6	0.32	5.48
						17.61	9.07	0.43	3.66	0.31	5.30
EL03	42° 14.01′	-8° 46.35′	new	2000E	36.25	15.59	10.30	0.41	3.91	0.36	5.64
						22.82	10.18	0.29	4.17	0.36	5.50
EL06	42° 12.82′	−8° 49.01′	new	2000W	37.40	14.05	9.03	0.48	3.4	0.29	5.48
						22.91	8.91	0.4	3.35	0.28	5.28

(Suppl. Fig. 2).

3.2. Chemical composition in the sediment: trace elements, PAHs, PCBs and OCPs

The concentrations of Cd, Cr, Cu, Hg, Pb and Zn, the metals with the highest ecotoxicological relevance, are shown in Fig. 2. Regarding Cd, Hg, and Pb, the BAC indicates that all concentrations, with the exception of Hg, were below this SQC, and can be considered background values. For this latter metal, only concentrations in the proximities to the old outfall, and 2000W were below the limit. Nevertheless, concentrations at all sites decreased from year 2016 to 2017 for this metal. Even though the rest of the metals showed background concentrations, some tendencies were elucidated among sites and between years. Values for Cr, Cu, Pb and Zn in the proximities to the new outfall (100E and 100W) increased from 2016 to 2017, when this UWWTP started working. However, increasing concentrations between years were also observed for these metals in more distant sites, as for instance, Cr concentrations

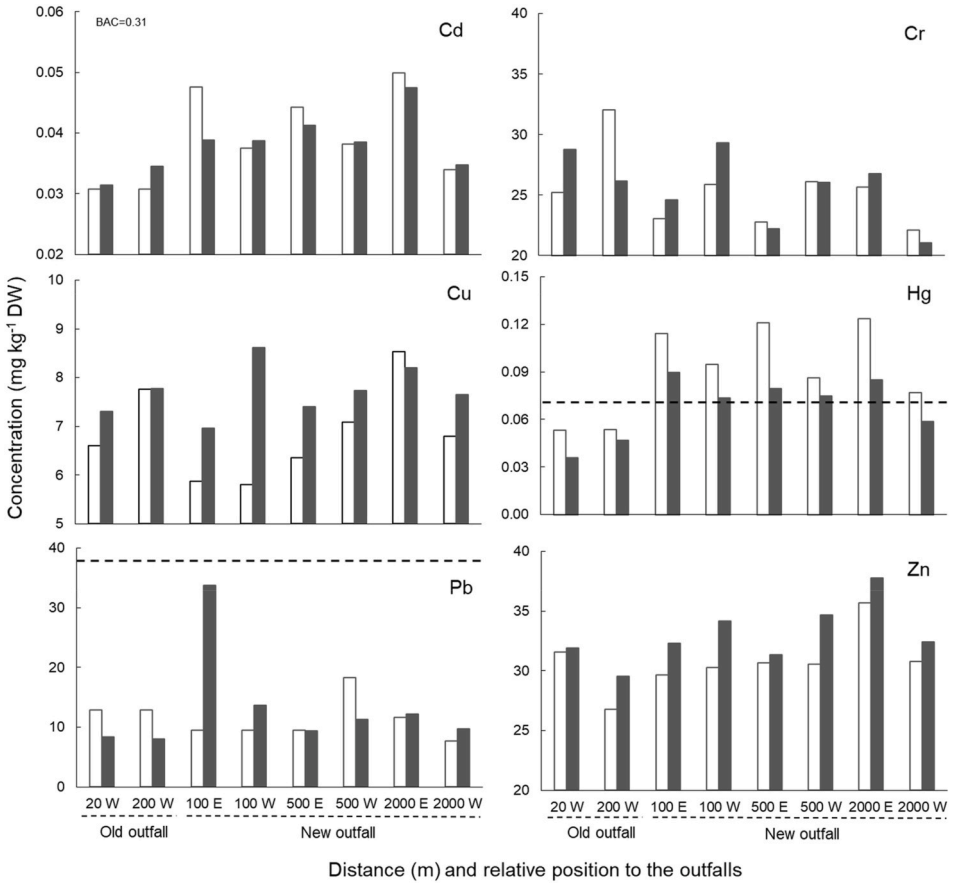

Fig. 2. Concentrations (mg kg^{-1} DW) of Cd, Cr, Cu, Hg, Pb and Zn in the sediment of the sites at Ria de Vigo in 2016 (open bars) and 2017 (grey bars). Concentrations are normalized to 5% Al. Dashed lines represent background assessment concentrations (BAC) (OSPAR Commission, 2010). Note that the Y-axis of the different panels differ.

at 100W or Zn concentrations at all sites. Higher concentrations for Cd, Cr and Cu, with the exception of Cr and Cu concentrations at 200W, were also observed in 2017 in the proximities to the old outfall, when this UWWTP was already not working. Contrary, Pb and Hg showed similar or lower concentrations in the latter year at the same sites. There is no clear decrease of concentrations with increasing distance from the outfalls, but at least Cd, Cr, Hg and Pb showed low concentrations at 2000W, the furthest site towards the ocean. Sites close to the new outfall (100E or 100W) showed the maximum concentrations of Cd, Cu, Hg, Pb and Zn (Fig. 2).

Cr, Hg, Ni and Pb SQGs for non-normalized data indicate that concentrations at almost all sites were above ERL at both years, with the exception of 20W and 200W for Hg, 200W for Ni, and at different sites for Pb (Suppl. Table 1). The greatest difference between years was actually observed in site 100E, as Pb concentrations were 3-fold higher in 2017 than in the previous year. Contrary, concentrations of Cd, Cu, and Zn were below these limits, with the exception of site 2000E that showed concentrations of Cu and Zn above the SQG.

Regarding changes in levels of PAHs, a remarkable increase in both LMW and HMW PAHs was observed in one of the sites closest to the new outfall (100E) in 2017, just after the new UWWTP started working (Fig. 3, Suppl. Table 2). Actually, maximum concentrations were recorded at that site for Phe (i.e., 146 μg kg^{-1} DW). Concentrations in the proximities to the old outfall were slightly higher in 2017 than in 2016, even though the UWWTP no longer worked that year. The rest of sites showed lower concentrations than in previously mentioned sites, and, in fact, concentrations were lower in 2017 than in 2016. Overall, concentrations of LMWPAHs, including Ant, Fla, Naph and Phe, were higher than concentrations of HMWPAHs.

The highest concentrations of PCBs were observed in 20W and 100E (~45.5 μg kg^{-1} DW) the closest sites to the outfalls (Table 2). Concentrations also increased towards the site 2000W (Suppl. Fig. 3). Congener CB101, identified as a priority marine pollutant, was the compound that contributed the most to these high values. The presence of OCPs was recorded in the sediments of all sites at both years (Fig. 4, Suppl. Fig. 4). As PCBs, OCPs showed the highest concentrations at 20W and 100E, and at 2000W. Concentrations of total OCPs at all sites, excepting the closest sites to the new outfall and 200W, were higher in 2017 than in 2016. Heptachlor-epoxide and endrin were the compounds that contributed the most to these high concentrations (Suppl. Fig. 4).

Fig. 5 shows the bidimensional configuration of the sampling sites in 2016 and 2017 according to nMDS and classification into groups by cluster analysis based on the concentrations of metals, PAHs, 7CBs and OCPs (Figs. 2–4). All sites close to the old outfall were grouped together

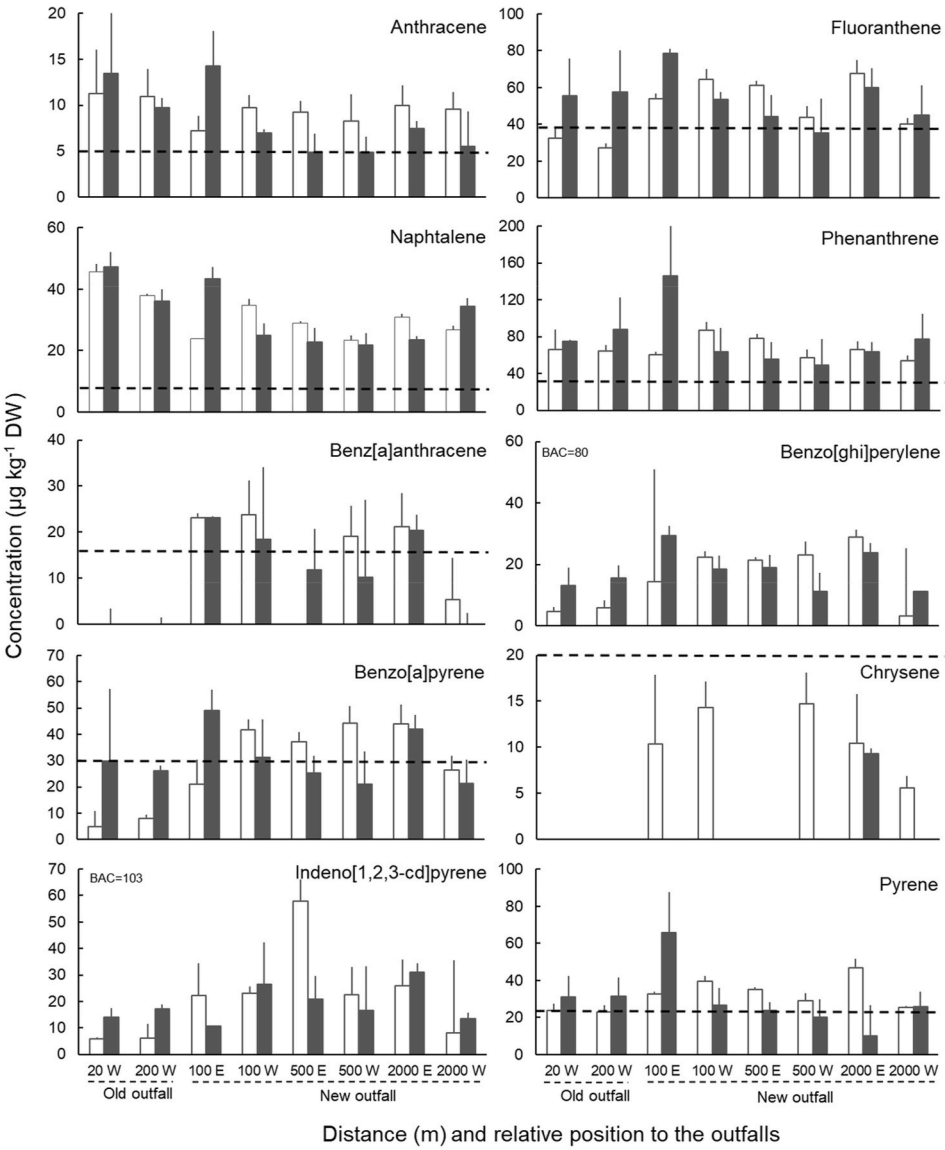

Fig. 3. Concentrations (mean ± sd, n = 3, μg kg^{-1} DW) of low molecular weight PAHs (anthracene, fluoranthene, naphthalene, and phenanthrene) and high molecular weight PAHs (benz[a]anthracene, benzo[gui]perylene, benzo [a]pyrene, chrysene, indenol[1,2,3-cd]pyrene, and pyrene) in the sediment of the sites at Ria de Vigo in 2016 (open bars) and 2017 (grey bars). Concentrations are normalized to 2.5% TOC. Dashed lines represent background assessment concentrations (BAC) (OSPAR Commission, 2010). Note that the Y-axis of the different panels differ.

Table 2
Concentration of polychlorinated biphenyls (mean ± sd, n = 3, μg kg^{-1} DW) in the sediment samples from Ria de Vigo in 2016 (first value) and 2017 (second value). Values are normalized to 2.5% TOC. Background assessment concentrations (BAC) and environmental assessment concentrations (EAC) for each congener are presented (OSPAR Commission, 2010). *: Congeners considered as priority marine pollutants by international agencies. The concentrations of the congeners No. 105, 118*, 138*, 149, 156, 187, 170 and 194 were below the detection limits at all sites. Values in bold show higher mean concentrations than EAC.

	BAC	EAC	Old outfall		New outfall					
			20 W	200 W	100 E	100 W	500 E	500 W	2000 E	2000 W
CB18			13.87 ± 0.42 17.75 ± 1.01	8.99 ± 0.53 8.61 ± 1.14	19.18 ± 1.12 6.38 ± 1.19	17.12 ± 1.71 6.36 ± 1.43	7.82 ± 0.98 8.72 ± 2.34	8.26 ± 1.99 7.24 ± 0.36	7.48 ± 0.23 10.98 ± 1.71	8.24 ± 1.06 14.98 ± 0.31
CB28*	0.22	1.7							**2.27 ± 2.35**	
CB31			14.97 ± 0.05 21.05 ± 0.33	8.62 ± 0.05 9.05 ± 0.12	19.99 ± 0.16 6.75 ± 0.06	13.86 ± 0.04 6.93 ± 0.05	7.15 ± 0.09 8.47 ± 0.08	6.05 ± 0.10 6.95 ± 0.10	7.19 ± 0.12 10.32 ± 0.11	8.09 ± 0.27 15.62 ± 0.14
CB44			2.94 ± 0.11 4.09 ± 0.08	1.6 ± 0.12 1.70 ± 0.08	5.37 ± 1.36 1.20 ± 0.04	2.45 ± 0.14 1.31 ± 0.09	1.40 ± 0.06 1.59 ± 0.14	1.08 ± 0.16 1.25 ± 0.02	1.25 ± 0.10 1.82 ± 0.06	1.72 ± 0.21 2.94 ± 0.03
CB52*	0.12	2.7	**2.83 ± 0.10** **4.8 ± 0.15**	1.92 ± 0.16 1.68 ± 0.15	**3.88 ± 0.16** 1.49 ± 0.08	1.31 ± 0.10	1.66 ± 0.15	1.18 ± 0.36 1.42 ± 0.18	1.77 ± 0.00	1.66 ± 0.27 **2.99 ± 0.12**
CB101*	0.14	3.0	**32.38 ± 0.21** **41.15 ± 0.56**	**17.62 ± 0.01** **18.74 ± 0.05**	**41.52 ± 0.07** **13.99 ± 0.08**	**28.82 ± 0.12** **14.96 ± 0.15**	**15.38 ± 0.16** **17.85 ± 0.10**	**12.44 ± 0.28** **14.27 ± 0.14**	**15.42 ± 0.24** **20.62 ± 0.10**	**16.9 ± 0.25** **32.64 ± 0.06**
CB153*	0.19	40							0.94 ± 0.43	
CB180*	0.10	12		1.02 ± 0.21					1.20 ± 0.27 0.84 ± 0.42	

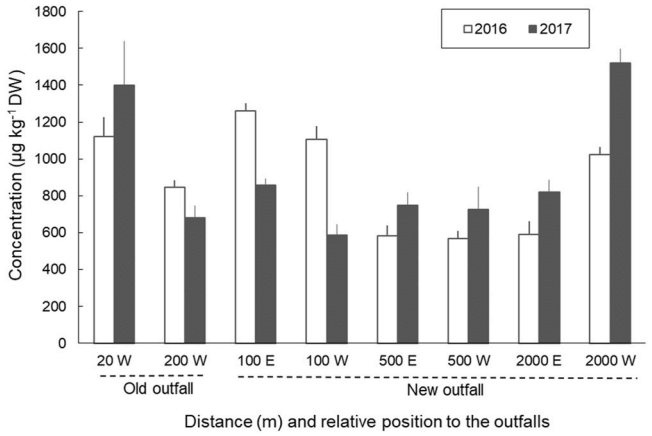

Fig. 4. Total organochlorine pesticides (OCPs) concentrations (mean ± sd, n = 3, μg kg^{-1} DW) in the sediment of the sampling sites at Ria de Vigo in 2016 (open bars) and 2017 (grey bars). Concentrations are normalized to 2.5% TOC.

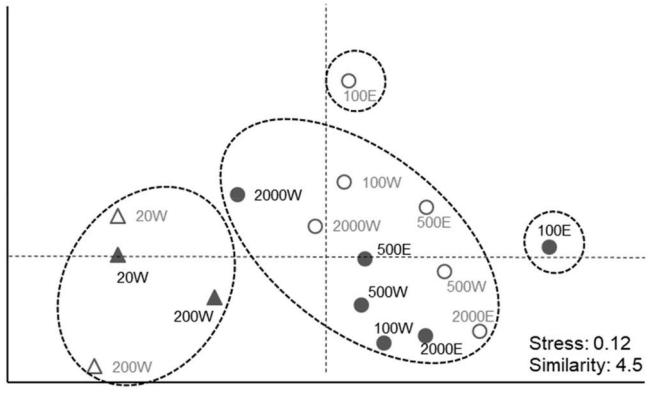

Fig. 5. Bidimensional configuration of the sampling sites at the old (triangles) and new (circles) outfalls at Ria de Vigo in 2016 (open symbols) and 2017 (grey symbols) obtained by non-metric multidimensional scaling (nMDS) according to environmental (silt/clay and OM content) and normalized chemical data (Cd, Cr, Cu, Hg, Pb, Zn, Σ LMWPAHs, Σ HMWPAHs, Σ 7CBs, and Σ OCPs). Groups from the hierarchical cluster analysis based on Euclidean distances are circled on the plot. Stress of the nMDS and similarity of the Cluster analysis are specified on each plot.

at both sampling years, showing that, based on the chemical composition of the sediment, the area did not totally recover in one year. On the contrary, 100E, the closest site to the new outfall was separated between years and segregated from the big group including sites 100W, and at 500 and 2000 m. Differences in chemical concentrations during the installation of the outfall and the latter functioning might have made the difference.

3.3. Biological responses

The highest toxicity showed by the sea urchin toxicity embryo-larval bioassay was observed in 20W, the closest site to the old outfall, in 2017 (Fig. 6) while sites more distant from the outfalls did not show any toxicity. The closest site to the old outfall (20W) increased its toxicity from moderate to bad in 2017. Contrary, the site 200W decreased its toxicity down to a good status in 2017. Sites closest to the new outfall, showed moderate or no toxicity in all cases.

Regarding the macrobenthic community, the area showed a high macrobenthic diversity with 134 taxons from 37 families (Table 3). The highest abundances occurred in 20W and 200W, the closest sites to the old outfall, in the year 2017, when the UWWTP had already stopped working. The lowest abundances were observed at 2000E, the site in the innermost area, with 531 individuals m^{-2} in the year 2017. In fact, the bidimensional configuration of the sites and classification into groups based on family abundance allowed the identification of 3 groups of sites segregating in one of them all sites closest to the old outfall (Fig. 7).

Univariate measures showed the macrobenthic community had time to recover from the year 2016 to 2017 in the proximities to the old outfall (Table 3). Contrary, sites close to the new outfall showed different trends, while evenness and diversity values were lower at 100E in 2017, values observed in 100W were higher in this same year. Therefore, communities at the same distance but at different positions were differentially affected. Sites located further than 500 m from the new outfall did not show any specific trend, being also grouped with the rest of the sites, with the exception of the site 500E (Fig. 7).

The community was dominated by the class Polychaeta and the most abundant families were Ampharetidae and Capitellidae (Suppl. Table 3).

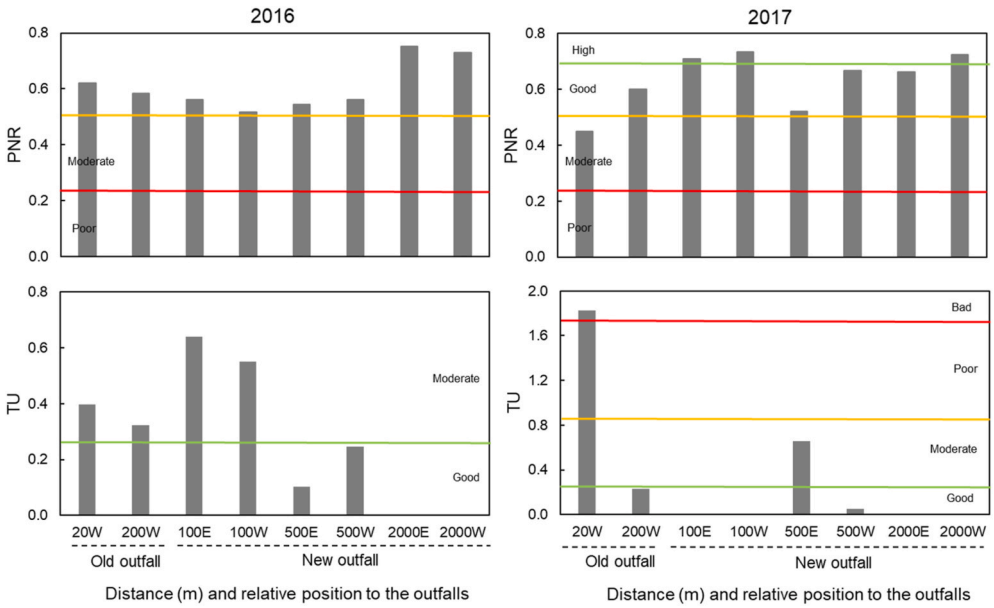

Fig. 6. Percentage net response (PNR) and toxic units (TU) of the sediment elutriates from the sampling sites at Ria de Vigo in 2016 (left panels) and 2017 (right panels). Horizontal lines limit the different ecological status in the toxicity assessment of each parameter (Durán and Beiras, 2010). Note that Y-axis of TU panels differ.

Table 3
Total number of families (S), abundance (N, individuals m^{-2}), Margalef's species richness index (d), Pielou's evenness index (J′) and Shannon-Wiener diversity index (H′) at the sampling sites in Ria de Vigo in 2016 (first value) and 2017 (second value).

Site	S	N	d	J'	H'
20 W	35	1556	6.15	0.74	2.62
	36	2704	5.75	0.78	2.79
200 W	35	1426	6.25	0.70	2.47
	37	1920	6.27	0.78	2.80
100 E	28	1630	4.84	0.75	2.50
	35	1586	6.13	0.67	2.37
100 W	36	1809	6.16	0.65	2.32
	34	1167	6.30	0.80	2.84
500 E	23	710	4.64	0.87	2.74
	34	920	6.59	0.87	3.06
500 W	30	1679	5.17	0.76	2.60
	25	1148	4.59	0.75	2.43
2000 E	26	895	5.02	0.84	2.75
	25	531	5.39	0.93	3.00
2000 W	22	951	4.17	0.64	1.98
	29	1833	4.92	0.77	2.59

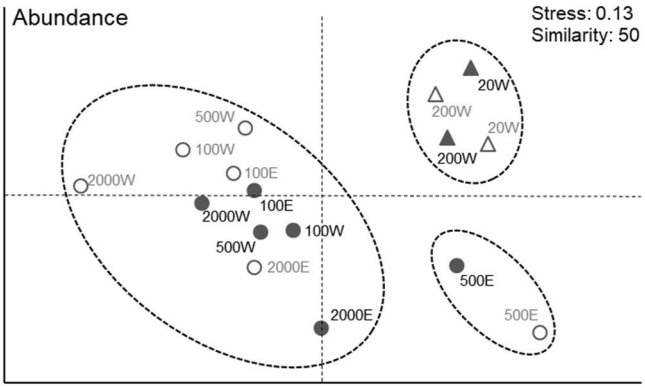

Fig. 7. Bidimensional configuration of the sites at the old (triangles) and new (circles) outfalls at Ria de Vigo in 2016 (open symbols) and 2017 (grey symbols) obtained by non-metric multidimensional scaling (nMDS) based on families' abundance data. Groups from the hierarchical cluster analysis based on Bray-Curtis similarities are circled on the plot. Stress of the nMDS and similarity of the Cluster analysis are specified.

The most remarkable changes in BOPA index (Fig. 8) were observed in sites 100W and 2000W, which values were 2.5 and 1.5-fold higher in 2017 respectively. In site 100W the ecological status changed from good to moderate. In all other cases interannual changes in BOPA ranged from 0.13 (good) to 0.22 (poor).

3.4. Relationships between biological responses and chemical results

Concentrations of organic chemicals, PAHs, 7CBs and OCPs, were significantly correlated with OM and silt/clay content (Table 4). Among biological responses, total abundance of benthic infauna was also negatively correlated with OM (*p-value*<0.01). H′, BOPA, or the toxicity results (estimated from the sea urchin toxicity embryo-larval bioassay) were not significantly correlated with these environmental variables.

Among biological responses, PNR, estimated from the sea urchin toxicity embryo-larval bioassay, was positively correlated with concentrations of Cu and Zn. In regard the indices estimated with the macrobenthic community composition data, neither H′ nor BOPA were correlated with environmental or chemical variables. Abundance was inversely, but weakly, correlated with Cd and Hg concentrations (r = −0.504 and −0532 respectively, *p-value*<0.05), but positively correlated with organic compounds such as LMWPAHs and 7CBs (r = 0.598 and 0.589 respectively, *p-value*<0.05).

Correspondence analysis results (Fig. 9) reflected that segregation of sites closest to the old outfall was related with high LMWPAHs and Cu concentrations, and low HMWPAHs and Hg concentrations and OM content. Sites in the surroundings to the new outfall, together with 2000E and 2000W, had in common high OM and silt/clay sediment content and high HMWPAHs concentrations. As previously shown by the abundance ordination (Fig. 7), site 500E falls apart from all other sites, also showing a different macrobenthic community. The same sites sampled at different years were plotted together, indicating that no big changes occurred between years.

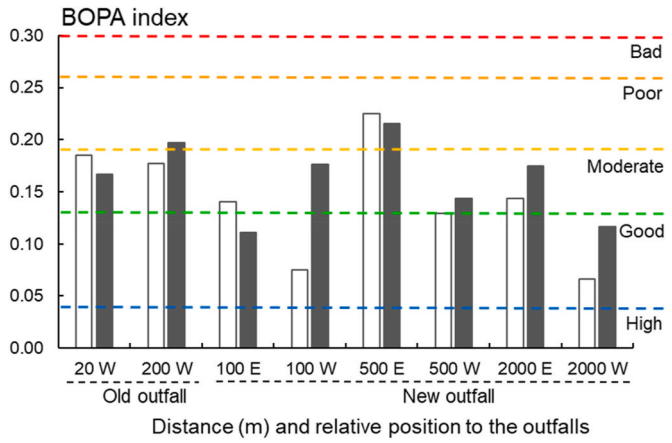

Fig. 8. Values for BOPA index from the sampling sites at Ria de Vigo in 2016 (open bars) and 2017 (grey bars). Horizontal lines limit the different ecological status assessment proposed by Dauvin and Ruellet (2007).

4. Discussion

Monitoring effluents from anthropogenic origin has usually been based either in chemical analysis on an abiotic matrix (Gerbersdorf et al., 2015) or on biological responses (Munkittrick et al., 2009). In this study we included chemical, toxicological and macrobenthic fauna data from the same sediment samples, providing a unique opportunity to link the environmental and chemical variables to the biological responses and highlight the potential limitations of all methods. In this sense, fewer studies have aimed to connect the different indexes used in monitoring studies related to UWWTPs (Martinez-Haro et al., 2015).

4.1. Main contaminants of the UWWTPs

The Ria de Vigo supports high population densities and different industrial activities, being the final deposit of many contaminants. Therefore, it could be somehow misleading to distinguish if stations are impacted by the UWWTPs or by other sources. In this sense, even though the sites were established as a gradient, the concentrations of certain contaminants did not decrease at increasing distance from the outfall. Actually, sites located at 2000 m showed high concentrations of OCPs or PCBs, with similar values to the ones at the closest sites to the outfalls. Therefore, all sites could suffer a certain degree of anthropogenic contamination from diffuse sources.

Changes in sediment grain size (i.e. proportion of silt/clay) constitute a limitation in assessing potential risks based on the chemical composition. To overcome this limitation, concentrations of metals and organic compounds were normalized by the Al and TOC content respectively. Concentrations of metals were overall below the BAC (OSPAR Commission, 2010). Contrary, organic compounds, such as PAHs and PCBs, were above these background values at almost all sites. Nevertheless, these BAC values are statistical tools defined in relation to the background (BC) and low concentrations (LC) (OSPAR Commission, 2010) which represent the concentrations at "remote" sites considered pristine. Although BAC could be reasonable predictive on establishing the existence of contamination, there is no ecological rationale behind this method, and consequences for the systems cannot be predicted.

In this way, the effects-based SQC for PAHs concentrations for the Galician rias (Bellas et al., 2011) or the ERL (MacDonald et al., 1996) could help in the interpretation of organic sediment chemistry data and

Table 4
Pearson correlation coefficients (lower semimatrix) and significance (upper semimatrix) between environmental and chemical composition of the sediment and the biological responses at the sites at Ria de Vigo. Green cells indicate positive correlations while red cells indicate inverse correlations. Significant values (*p-value:* *: <0.05, **: ≤0.01, ***: ≤0.001) appear in boldface.

	silt/clay	OM	Cd	Cu	Cr	Hg	Pb	Zn	Σ LMWPAH	Σ HMWPAH	Σ 7CBs	Σ OCPs	PNR	TU	N	H'	BOPA
silt/clay	-	**0.025**	0.408	0.560	0.367	0.116	0.460	0.112	0.597	**0.017**	**0.020**	0.250	0.199	0.173	0.385	0.476	0.065
OM	**0.557***	-	**0.001**	0.916	0.051	**0.000**	0.415	0.089	**0.047**	**0.003**	0.125	0.830	0.276	0.072	**0.007**	0.730	0.644
Cd	0.222	**0.738*****	-	0.839	0.698	**0.001**	0.567	**0.037**	0.109	**0.002**	0.651	0.535	0.642	0.398	**0.047**	0.305	0.938
Cu	0.157	-0.029	0.055	-	0.107	0.317	0.934	**0.030**	0.622	0.761	0.170	0.084	**0.043**	0.284	0.353	0.060	0.275
Cr	0.226	-0.496	-0.105	0.418	-	0.094	0.713	0.832	0.360	0.532	0.797	0.116	0.585	0.400	0.419	0.571	0.425
Hg	0.409	**0.834*****	**0.768*****	-0.267	-0.433	-	0.138	0.184	0.184	**0.002**	0.273	0.627	0.622	0.181	**0.034**	0.801	0.534
Pb	0.199	0.219	0.155	0.023	0.100	0.610	-	0.100	0.084	**0.044**	0.188	0.255	0.280	0.296	0.925	0.566	0.421
Zn	0.412	0.438	**0.525***	**.543***	-0.058	0.494	0.713	-	0.432	0.165	0.642	0.622	**0.048**	0.293	0.102	0.160	0.897
Σ LMWPAH	-0.143	**-0.502***	-0.416	-0.133	0.245	-0.350	0.445	-0.211	-	0.708	0.374	0.192	0.596	0.198	**0.014**	0.706	0.854
Σ HMWPAH	**0.586***	**0.695****	**0.712****	-0.083	-0.169	**0.717****	**0.509***	0.365	0.101	-	0.353	0.860	0.729	0.552	0.537	0.749	0.330
Σ 7CBs	**-0.572***	-0.400	-0.123	-0.361	-0.070	-0.292	-0.347	-0.126	0.238	-0.249	-	**0.041**	0.138	**0.005**	**0.016**	0.895	0.832
Σ OCPs	-0.305	-0.058	-0.167	-0.445	-0.409	-0.132	0.302	-0.133	0.344	-0.048	**0.516***	-	0.873	0.600	0.347	0.227	0.165
PNR	0.339	0.290	0.126	**0.512***	-0.148	0.133	0.287	**0.500***	-0.143	0.094	-0.388	0.044	-	**0.000**	0.091	0.391	0.186
TU	-0.358	-0.461	-0.227	-0.285	0.226	-0.352	-0.278	-0.280	0.339	-0.161	**0.669****	0.142	**-0.788****	-	**0.005**	0.479	0.506
N	-0.233	**-0.643****	**-0.504***	-0.249	0.217	**-0.532***	0.026	-0.424	**0.598***	-0.167	**0.589***	0.252	-0.437	**0.670****	-	0.607	0.533
H'	-0.192	0.094	0.274	0.479	0.153	-0.068	-0.155	0.368	-0.102	0.087	0.036	-0.320	-0.230	0.191	-0.139	-	**0.000**
BOPA	-0.472	-0.125	-0.021	0.290	0.214	-0.168	-0.216	-0.035	-0.050	-0.260	-0.058	-0.364	-0.348	0.180	-0.169	**0.802****	-

OM: organic matter; Σ LMWPAH: sum of low molecular weight PAHs; Σ HMWPAH: sum of high molecular weight PAHs; PNR: predictive n

bioassay, N: abundance, H': Shannon-Wiener diversity index.

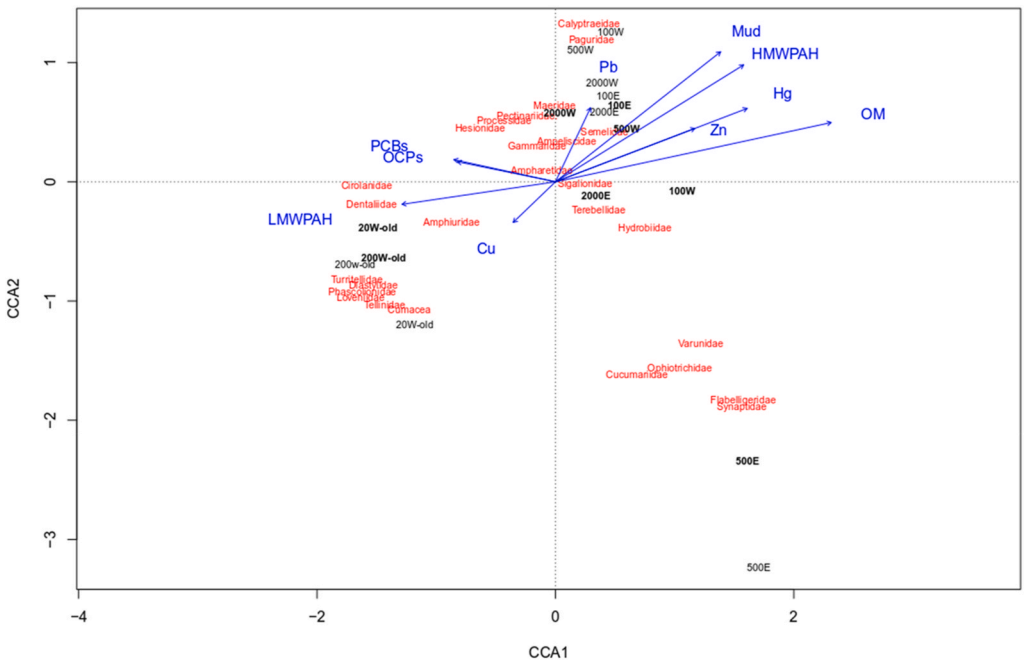

Fig. 9. Canonical correspondence analysis ordination biplot for the most abundant families of the macrobenthic communities at Ria de Vigo (Suppl. Table 3). Response variables (families' abundance) are in red. Objects (sites) are represented in black (2016) and in bold (2017). Arrows represent the direction and strength (length) of quantitative explanatory variables (sediment variables) with arrowheads indicating their direction of increase. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

to identify potential pollutants as indicators of old and new UWWTPs. These criteria represent concentrations above which adverse effects would occasionally occur, as were frequently exceeded with no toxic effects on the sea-urchin embryo-larval bioassay (Bellas et al., 2011). On the basis of the concentrations observed at Ria de Vigo, the ERL values, such as the TEL, highlights the higher impact of LMWPAHs compared to HMWPAHs. Therefore, sites in the surroundings of the outfalls were mainly impacted by organic compounds, including OCPs that were banned decades ago. The trends observed for the organic compounds suggest that although both the old and new UWWTPs are related with high concentrations, there exist other sources altering the natural levels of these compounds in the Ria de Vigo.

But overall, organic compounds such as LMWPAHs, PCBs and OCPs, mainly characterized the sites close to the old outfall. While the new outfall was mainly characterized by metal concentrations and HMWPAHs. The similar ordination of the same sites at the different years shows that the sites close to the old outfall did not have time to totally recover in one year. On the other side, during 2016, even though the new outfall was not working, the construction had already started, causing a disturb in the sediment that was clear by the separated ordination of the site 100E.

Chemical concentrations observed in this study are within the range of previously reported values in the Ria de Vigo (Beiras et al., 2003a; Bellas et al., 2011; Quelle et al., 2011) with the exception of the harbor area (Montero et al., 2013). These areas, as the inner parts of the rias show the highest anthropogenic impacts, since they present the highest levels of contaminants with more harmful effects to marine organisms as shown in different studies carried out during the last years (Bellas et al., 2011; Beiras et al., 2012b; Montero et al., 2013). In this sense, the position of the outfalls, at the outer zone of the ria, might help to dissipate the potential pollutants.

$\delta^{15}N$ is a tracer that delimits the intensity and impact area of anthropogenic inputs, including sewage outfalls (Deutsch et al., 2006; Dailer et al., 2012). The impact and recovery of the old outfall could be tracked by means of changes in the N stable isotopes. Contrary, the $\delta^{15}N$ values close to the new outfall were not different from the ones observed in the rest of the sites (5.4–5.6‰) considered as background values (Savage, 2005). The improvement in the removal of N and residual organics in the new UWWTP could be the reason for this lack of impact, as previously observed. Therefore, the question if $\delta^{15}N$ can be used to track the impact of modern UWWTP arises again (Viana and Bode, 2013).

4.2. *Integrative assessment of the ecological status of sites at Ria de Vigo*

BOPA classified the sites at Ria de Vigo from poor, site 500E, to good ecological status, including both the closest and the furthest sites to the new outfall. This means that even though the concentrations of some chemicals were above the SQC, the ecological effects at a community level are not that meaningful.

As shown by the macrobenthic community indices, one year is enough for the recovery of the community in terms of abundance, evenness and diversity. However, BOPA index and chemical concentrations in the sediment indicated that recovery was not complete. Nevertheless, the status of the furthest sites from both outfalls at different years were also classified as moderate, indicating that, even though there is a local impact of the UWWTPs, the anthropogenic pressure on the ria is moderate.

While BOPA and diversity indices has been successfully used in many studies (Spagnolo et al., 2014; Dauvin, 2018) different authors highlight the existence of certain limitations related with them. In this study, neither BOPA nor H' were correlated with any of the chemicals analyzed, while family abundance was correlated with Cd, Hg, LMWPAHs and 7CBs concentrations, as well as with the toxicity test results from the sea urchin embryo-larval bioassay. Abundance also segregated the sites closer to the old outfall, and so did the concentration of the different chemicals. Therefore, its use in the present study was more useful than BOPA or diversity indices.

For instance, benthic community composition and abundance of families at site 500E was significantly different from the other sites. The toxicity results from the sea urchin embryo-larval bioassay showed that the ecological status at that site was slightly poorer than the others, discarding the sediment characteristics as a possible cause of such difference. Contrary, concentrations of the chemicals analyzed in the sediment were not high at that site, suggesting that the presence of some other chemical or other environmental condition not considered might affect the ecological status at that site and hence affect the community composition.

4.3. Limitations of the different assessment criteria: is there "a best indicator" of UWWTPs?

Chemical determination in sediments has allowed us to detect high concentrations of certain pollutants. But, while BAC are arbitrary thresholds with no ecological background, ERL or local SQC limit the comparison of sites with different granulometric characteristics. The higher surface:volume ratio of fine particles (silt/clay) and the specific surface properties of organic particles present a higher capacity of retention of chemical contaminants that had the capacity to stick on their surface (Yariv and Cross, 1979; Bradford and Horowitz, 1982). Also, the traditional chemical analysis limits the study to the known contaminants. Nowadays with the emerging contaminants that are being detected, covering the spectrum of potential contaminants is becoming difficult. In this study, in spite of the wide spectrum of chemicals analyzed, some ecological responses (e.g. the site 500E) could not be explained, as well as the potential interactive effects of the mixture of pollutants.

On the other side, indices related with macrobenthic infaunal assemblages discriminated between stations differentially affected by the outfalls at Ria de Vigo, but those differences could be related to both anthropogenic or environmental variables. Macrobenthic marine communities have been successfully used to assess contamination but changes could also be related to the grain size characteristics. And, actually, according to our analysis, the faunistic composition at the different sites was mainly influenced by the OM content and LMWPAHs concentrations.

As a paradox, even though chemical concentrations observed in some sites were above the assessment criteria, toxicity tests only highlighted the toxicity in the proximities to the old outfall, while no toxic effects were observed in the proximities to the new outfall.

5. Conclusions

Significant segregation of the sites close to the old outfall based on chemical composition and abundance of the macrobenthic community shows that the upgrade in the depuration process of the new UWWTP could be assessed based on both chemical and ecological evaluation.

Ecological responses, such as community level changes and the sea urchin embryo-larval bioassay seem to complementary reinforce the need to use combined approaches in order to get a better assessment of submarine sewage impact. Nevertheless, bioassays seem to be an optimal tool in terms of cost-benefit in the ecological assessment.

CRediT authorship contribution statement

Inés G. Viana: Writing - original draft. **Raimundo Blanco:** conceived and designed the sampling design. All authors contributed to the article and approved the final version. **Óscar Nieto:** Formal analysis, performed the PAHs, PCBs and OCPs analysis, All authors contributed to the article and approved the final version. **Alberto Molares:** Formal analysis, performed the PAHs, PCBs and OCPs analysis, All authors contributed to the article and approved the final version. **Ricardo Beiras:** conceived and designed the sampling design. All authors contributed to the article and approved the final version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Acknowledgements

Chemical analysis was done at the Centro de Apoyo Científico y Tecnológico a la Investigación (CACTI) of the University of Vigo, with the exception of the N isotopic analysis that was carried out at the Unidade de Técnicas Instrumentais de Análise (University of A Coruña) and organic compounds analysis that was performed at the Óscar Nieto lab at Analytical and Food Chemistry Department (University of Vigo). Inés Viana was awarded with a postdoctoral fellowship of Xunta de Galicia (Consellería de Educación, Universidad e Formación Profesional) postdoctoral programme (ED481B-2016/189-0). We thank Pilar Feijóo and Tania Tato for their technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2020.105234.

References

Beiras, R., Bellas, J., Fernández, N., Lorenzo, J.I., Cobelo-García, A., 2003a. Assessment of coastal marine pollution in Galicia (NW Iberian Peninsula); metal concentrations in seawater, sediments and mussels (*Mytilus galloprovincialis*) versus embryo-larval bioassays using *Paracentrotus lividus* and *Ciona intestinalis*. Mar. Environ. Res. 56, 531–553.

Beiras, R., Fernández, N., Bellas, J., Besada, V., González-Quijano, A., Nunes, T., 2003b. Integrative assessment of marine pollution in Galician estuaries using sediment chemistry, mussel bioaccumulation, and embryo-larval toxicity bioassays. Chemosphere 52, 1209–122.

Beiras, R., Durán, I., Bellas, J., Sánchez-Marín, P., 2012a. Biological effects of contaminants: *Paracentrotus lividus* sea urchin embryo test with marine sediment elutriates. ICES Tech. Mar. Environ. Sci. 51, 13.

Beiras, R., Durán, I., Parra, S., Urrutia, M.B., Besada, V., Bellas, J., et al., 2012b. Linking chemical contamination to biological effects in coastal pollution monitoring. Ecotoxicology 21, 9–17.

Beiras, R., Durán, I., 2014. Objective classification of ecological status in marine water bodies using ecotoxicological information and multivariate analysis. Environ. Sci. Pollut. Res. 21, 13291–13301.

Bellas, J., Nieto, Ó., Beiras, R., 2011. Integrative assessment of coastal pollution: development and evaluation of sediment quality criteria from chemical contamination and ecotoxicological data. Continent. Shelf Res. 31, 448–456.

Braak, C.J.F.T., 1986. Canonical correspondence analysis: a new Eigenvector technique for multivariate direct gradient analysis. Ecology 67, 1167–1179.

Bradford, W.L., Horowitz, A.J., 1982. The Role of Sediments in the Chemistry of Aquatic Systems: Proceedings of the Sediment Chemistry Workshop, 969. U.S. Government Printing Office.

Clark, K.R., Warwick, R.M., 1994. Change in Marine Communities: an Approach to Statistical Analysis and Interpretation. Natural Environment Research Council, UK.

Dailer, M.L., Ramey, H.L., Saephan, S., Smith, C.M., 2012. Algal $\delta^{15}N$ values detect a wastewater effluent plume in nearshore and offshore surface waters and three-dimensionally model the plume across a coral reef on Maui, Hawai'i, USA. Mar. Pollut. Bull. 64, 207–213.

Dauvin, J.-C., 2000. The muddy fine sand *Abra alba-Melinna palmata* community of the Bay of Morlaix twenty years after the *Amoco Cadiz* oil spill. Mar. Pollut. Bull. 40, 528–536.

Dauvin, J.C., Ruellet, T., 2007. Polychaete/amphipod ratio revisited. Mar. Pollut. Bull. 55, 215–224.

Dauvin, J.C., Bellan, G., Bellan-Santini, D., 2010. Benthic indicators: from subjectivity to objectivity–Where is the line? Mar. Pollut. Bull. 60, 947–953.

Dauvin, J.-C., 2018. Twenty years of application of Polychaete/Amphipod ratios to assess diverse human pressures in estuarine and coastal marine environments: a review. Ecol. Indicat. 95, 427–435.

de-la-Ossa-Carretero, J.A., Del-Pilar-Ruso, Y., Giménez-Casalduero, F., Sánchez-Lizaso, J. L., Dauvin, J.-C., 2012a. Sensitivity of amphipods to sewage pollution. Estuar. Coast Shelf Sci. 96, 129–138.

de-la-Ossa-Carretero, J.A., Simboura, N., Del-Pilar-Ruso, Y., Pancucci-Papadopoulou, M. A., Giménez-Casalduero, F., Sánchez-Lizaso, J.L., 2012b. A methodology for applying taxonomic sufficiency and benthic biotic indices in two Mediterranean areas. Ecol. Indicat. 23, 232–241.

Del-Pilar-Ruso, Y., de-la-Ossa-Carretero, J.A., Giménez-Casalduero, F., Sánchez-Lizaso, J. L., 2007. Spatial and temporal changes in infaunal communities inhabiting soft-bottoms affected by brine discharge. Mar. Environ. Res. 64, 492–503.

Del-Pilar-Ruso, Y., de-la-Ossa-Carretero, J.A., Giménez-Casalduero, F., Sánchez-Lizaso, J. L., 2008. Effects of a brine discharge over soft bottom Polychaeta assemblage. Environ. Pollut. 156, 240–250.

Del-Pilar-Ruso, Y., de-la-Ossa-Carretero, J.A., Giménez-Casalduero, F., Sánchez-Lizaso, J. L., 2010. Sewage treatment level and flow rates affect polychaete assemblaes. Mar. Pollut. Bull. 60, 1930–1938.

Deutsch, B., Kahle, P., Voss, M., 2006. Assessing the source of nitrate pollution in water using stable N and O isotopes. Agron. Sustain. Dev. 26, 263–267.

Durán, I., Beiras, R., 2010. Assessment criteria for using the sea-urchin embryo test with sediment elutriates as a tool to classify the ecotoxicological status of marine water bodies. Environ. Toxicol. Chem. 29, 1192–1198.
Fernández, N., Beiras, R., 2001. Combined toxicity of dissolved mercury with copper, lead and cadmium on embryogenesis and early larval growth of the *Paracentrotus lividus* sea-urchin. Ecotoxicology 10, 263–271.
Field, J.G., Clarke, K.R., Warwick, R.M., 1982. A practical strategy for analyzing multispecies distribution patterns. Mar. Ecol. Prog. Ser. 8, 7–52.
Gerbersdorf, S.U., Cimatoribus, C., Class, H., Engesser, K.-H., Helbich, S., Hollert, H., et al., 2015. Anthropogenic trace compounds (ATCs) in aquatic habitats — Research needs on sources, fate, detection and toxicity to ensure timely elimination strategies and risk management. Environ. Int. 79, 85–105.
Kruskal, J.B., Wish, M., 1978. Multidimensional Scaling. Sage University Paper Series on Quantitative Applications in the Social Sciences, No. 07-011. Sage Publications, Newbury Park.
Kupper, T., Plagellat, C., Brändli, R.C., de Alencastro, L.F., Grandjean, D., Tarradellas, J., 2006. Fate and removal of polycyclic musks, UV filters and biocides during wastewater treatment. Water Res. 40, 2603–2612.
Long, E.R., MacDonald, D.D., Smith, S.L., Calder, F.P., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediment. Environ. Manag. 19, 81–97.
MacDonald, D.D., Carr, R.S., Calder, F.D., Long, E.R., Ingersoll, C.G., 1996. Development and evaluation of sediment quality guidelines for Florida coastal waters. Ecotoxicology 5, 253–278.
Marin, A., Montoya, S., Vita, R., Marín-Guirao, L., Lloret, J., Aguado, F., 2007. Utility of sea urchin embryo–larval bioassays for assessing the environmental impact of marine fishcage farming. Aquaculture 271, 286–297.
Martinez-Haro, M., Beiras, R., Bellas, J., Capela, R., Coelho, J.P., Lopes, I., et al., 2015. A review on the ecological quality status assessment in aquatic systems using community based indicators and ecotoxicological tools: what might be the added value of their combination? Ecol. Indicat. 48, 8–16.
Montero, N., Belzunce-Segarra, M.J., Menchaca, I., Garmendia, J.M., Franco, J., Nieto, O., et al., 2013. Integrative sediment assessment at Atlantic Spanish harbours by means of chemical and ecotoxicological tools. Environ. Monit. Assess. 185, 1305–1318.
Mucha, A.P., Vasconcelos, M.T.S.D., Bordalo, A.A., 2003. Macrobenthic community in the Douro estuary: relations with trace metals and natural sediment characteristics. Environ. Pollut. 121, 169–180.
Munkittrick, K.R., Arens, C.J., Lowell, R.B., Kaminski, G.P., 2009. A review of potential methods of determining critical effect size for designing environmental monitoring programs. Environ. Toxicol. Chem. 28, 1361–1371.
Nieto, Ó., Aboigor, J., Buján, R., N'Diaye, M., Graña, J., Saco-Álvarez, L., et al., 2006. Temporal variation in the levels of polycyclic aromatic hydrocarbons (PAHs) off the Galician Coast after the 'Prestige' oil spill. Mar. Ecol. Prog. Ser. 328, 41–49.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al., 2019. Vegan: Community Ecology Package. R Package Version 2.5-5.
OSPAR Commission, 2010. Agreement on CEMP Assessment Criteria for the QSR 2010 (OSPAR Agreement 2009-2). OSPAR Commission, London.
Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. 16, 229–311.
Quelle, C., Besada, V., Andrade, J.M., Gutiérrez, N., Schultze, F., Gago, J., et al., 2011. Chemometric tools to evaluate the spatial distribution of trace metals in surface sediments of two Spanish rías. Talanta 87, 197–209.
R Studio Team, 2018. RStudio. Integrated Development for R. RStudio, Inc, Boston, MA. http://www.rstudio.com.
Ríos, A.F., Nombela, M.A., Pérez, F.F., Rosón, G., Fraga, F., 1992. Calculation of runoff to an estuary. Ría de Vigo. Sci. Mar. 56, 29–33.
Savage, C., 2005. Tracing the influence of sewage nitrogen in a coastal ecosystem using stable nitrogen isotopes. Ambio 34, 145–150.
Smedes, F., de Boer, J., 1997. Determination of chlorobiphenyls in sediments-analytical methods. Trends Anal. Chem. 16, 503–517.
Spagnolo, A., Punzo, E., Santelli, A., Scarcella, G., Strafella, P., Grati, F., et al., 2014. Offshore platforms: comparison of five benthic indicators for assessing the macrozoobenthic stress levels. Mar. Pollut. Bull. 204, 55–65.
Viana, I.G., Bode, A., 2013. Stable nitrogen isotopes in coastal macroalgae: geographic and anthropogenic variability. Sci. Total Environ. 443, 887–895.
Warwick, R.M., Clarke, K.R., Suharsono, 1990. A statistical analysis of coral community responses to the 1982–83 El Niño in the Thousand Islands, Indonesia. Coral Reefs 8, 171–179.
Wells, D., Cofino, W., 1997. The assessment of the QUASIMEME laboratory performance studies data: techniques and approach. Mar. Pollut. Bull. 35, 18–27.
Yariv, S., Cross, H., 1979. Geochemistry of Colloid Systems. Springer-Verlag Berlin Heildelberg.
Zar, J.H., 2007. Biostatistical Analysis. Prentice-Hall, Inc., Upper Saddle River, NJ, USA.